



Diversity and abundance of airborne fungal spores in a rural cold dry desert environment in Argentinean Patagonia

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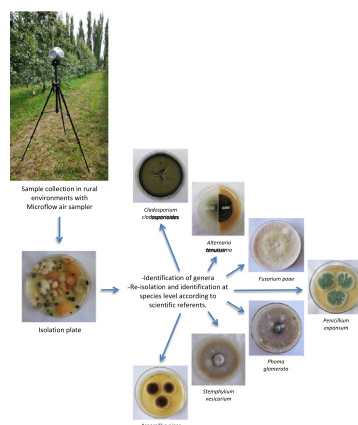
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HIGHLIGHTS

- This is the first longitudinal study of three consecutive years in rural environments with Köppen Bwk.
- The annual mean fungal counts were found in the order of $E+03$ CFU/m³ of air.
- The aerial mycoflora revealed at least 28 genera and 50 species in the sampling period.
- *Cladosporium* was the most abundant genus followed by *Alternaria* and *Epicoccum*.
- *C. cladosporioides*, *C. limoniforme* and *A. tenuissima* predominated.

GRAPHICAL ABSTRACT



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ABSTRACT

This work describes a longitudinal study of three consecutive years carried out in the air of agricultural environments located in Northern Patagonia with cold dry desert climate (Köppen: Bwk). This study area comprises a rural valley with unique geographical and climatological conditions. Therefore, the aim of this work is to quantify and determine its fungal diversity, so this knowledge will contribute to detect potential pathogenic and toxic fungi that has been adapted to this type of environment and may overcome the incipient climate change. Samplings were conducted in two geographical zones of the study area and a microflow air sampler was used to isolate fungal taxa. The annual mean fungal counts were found in the order of $E+03$ CFU/m³ of air. The aerial mycoflora revealed a wide biodiversity of at least 28 genera and 50 fungal species. *Cladosporium* was the most abundant genus (76.97%), followed by *Alternaria* (12.48%), *Epicoccum* (4.41%) and *Botrytis* (1.81%). The rest of the genera were found in relative densities lower than 1%. In terms of species, *C. cladosporioides* (34.82%), *C. limoniforme* (21.72%), *A. tenuissima* (10.94%) and *C. asperulatum* predominated (9.01%). This is the first report of the air mycoflora of rural environments with cold dry desert climate which provides useful information to take preventive measures to avoid biological damage.

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1. Introduction

The atmosphere does not contain an autochthonous microbiota, but it is a means for the dispersion of many types of microorganisms (De la Rosa et al., 2002). Aeromycological studies have been carried out in many parts of the world and they provide extremely important information due to the effects that fungal spores can cause on human, animal and plant health (Abdel Hameed et al., 2009; Reyes et al., 2009; Oliveira et al., 2010). Therefore, to systematically evaluate the relationship between environmental fungi and their adverse effects, the types of fungi and their concentration need to be known.

Some of these studies were conducted in urban areas and describe and quantify the fungal biodiversity of indoor (Cabral, 2010; Newbound et al., 2010; Khan and Karuppaiyil, 2012; Mentese et al., 2012; Sharpe et al., 2015) and outdoor environments (Curtis et al., 2006; Fröhlich-Nowoisky et al., 2009; Lang-Yona et al., 2016). Other studies have been performed in rural areas mainly for agricultural and phytopathological purposes (Pepeljnjak and Šegvić, 2003; Oliveira et al., 2009; Lanier et al., 2010; Temperini et al., 2018). Annually, crop losses due to fungal diseases reach large sums, causing big quantitative and/or qualitative economic losses to the productive sectors (Kasprzyk, 2008; Kakde, 2012). Therefore, the atmosphere is the most common means for the dispersion of fungal spores, the determination of the aerial mycobiota of a region, particularly its phytopathogenic fraction, provides useful information to take preventive measures contributing to improve productivity and health of the crops (Isard et al., 2007).

Studies conducted in the field of environmental mycology suggest that the fungal composition of air, in terms of concentration and composition of genera and species, varies with respect to geographical areas and is influenced by seasonality and other environmental factors (Reyes et al., 2009; Fröhlich-Nowoisky et al., 2012). Furthermore, the ongoing climate change can profoundly affect the production and dispersal of fungal spores, with the consequent modification of their air concentration and associated impact. Therefore, the monitoring of fungal spores in the air can be useful to detect spatial and temporal profiles of a changing climate (Beggs, 2010; Boccacci et al., 2017).

The High Valley of Río Negro is a rural valley located in the northern sector of the Argentinean Patagonia whose main activity is the growth and exportation of pip fruits. This agricultural environment presents a cold dry desert climate and it is classified in the BWk type of Köppen, cold dry desert climate with warm summers (Köppen, 1931). This study area presents unique geographical and climatological conditions (see Section 2.1 sampling area) which, in the context of the global climate change, may turn it into an extreme environment. Therefore, the aim of this work is to determine the annual concentration, density and distribution of the mycological diversity of this particular type of agricultural environment in order to detect potential pathogenic and toxic fungi that has been already adapted to these conditions and may overcome the incipient climate change. Results will allow to take preventive measures in order to avoid biological damage.

2. Material and methods

2.1. Sampling area

The High Valley of Río Negro is a rural valley located in the northern sector of the Argentinean Patagonia, between 38° 40' and 39° 20' S and 65° 50' and 68° 20' W in the confluence of three rivers. This "Y" shaped valley is arranged latitudinally and reaches a 100 km extension approximately. Its main economic activity is related to pip fruit production and it is the most important producing and exporting region of the nation. It is included in the climatic classification of Köppen in the BWk type (Köppen, 1931). The zone is characterized by a mean annual temperature of 15 °C and a marked thermal amplitude. The mean annual relative humidity is 65% and the mean annual rainfall is 243.7 mm. However, large variations are observed from year to year, characteristic of arid

climates. The wind is a factor of importance in the area due to its constancy and intensity, reaching gusts up to 74 km/h and its presence makes it necessary to install protective curtains, mainly malls. Due to its geographical location, the region has high values of global radiation with a mean annual value of 391.4 cal·gr/cm²·day (Rodríguez and Muñoz, 2006).

Due to its latitudinal extension, this valley could be divided into three equidistant zones: west, central and east. Even though weather characteristics are homogeneous along the valley, the eastern zone has presented, more often, outbreaks of apple and pear scabs which are related to high conditions of humidity (National Institute of Agricultural Technology -INTA-, personal communication). In terms of vegetation, the central zone has a higher density of rural settings (National Service for Agri-Food Health and Quality -SENASA-, SENASA, 2017). Therefore, eight rural settings were selected, four of them located in the eastern zone and the other four in the central zone separated by a distance of 50 km approximately (Fig. 1). All of them presented the spalter production system for pip fruits, currently the most widespread in the region. Regarding population, the eastern zone contains five towns and 50,191 habitants and the central zone contains 4 towns with 126,873 according to the last census (National Institute of Statistics and Censuses of the Argentine Republic-INDEC, 2010).

2.2. Sampling method

As we have not found previous information and/or reference of sampling methods to follow in large rural external environments, before starting this work, we carried out a sampling test in one of the selected rural settings in order to set methodology and all the parameters that would make the sampling campaigns reproducible such as volume and speed of sampling and culture medium.

Samplings were conducted using a microflow air sampler (Microflow α 90 Aquaria version 3.0.0 cod. G.1015) containing 90 mm disposable Petri dishes that allows to sample volumes of air up to a maximum of 2.000 L at five different speeds: 30 L/min, 60 L/min, 90 L/min, 100 L/min and 120 L/min. The sampler was placed at a height of 1.50 m above the ground level, on a photographic tripod and the head of the equipment was cleaned with isopropanol between each sampling. It was not used a positive hole conversion table to obtain data with this microflow air sampler.

First, we tried the medium Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Shelton et al., 2002), using air volumes of 40 L, 100 L and 180 L in order to include two extremes and one intermediate point of the sampler's range of volumes. Each volume was taken at three different speeds: 30 L/min, 90 L/min and 120 L/min.

The same test was then carried out with Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (0.1 g/L) to inhibit bacterial growth (Muhsin and Adlan, 2012). Due to the fact that in some of these plates invading fungi were observed, dichloran (2 mg/L) was added to the medium in the same concentration used in the DRBC medium (Pitt and Hocking, 2009) and the assay was repeated.

We observed that DRBC is more efficient to control the diameters of the colonies but it makes it difficult to observe some of the genera and their pigments when performing differential counting. Therefore, the culture medium of choice was PDA supplemented with chloramphenicol (0.1 g/L) and dichloran (2 mg/L).

With respect to the selection of sample volume and speed, the results yielded excessively high counts using volumes of 100 L or more at speeds >30 L/min. In addition, a high variability of counts was obtained at different geographical points of the rural setting in the same sampling day using the same volume and speed, so instead of taking replicas it was decided to take samples at each geographical point using two different volumes: 50 L and 100 L at the lowest speed of the sampler, 30 L/min. The selection of the plate per geographical point was made by the best distribution of the colonies for counting, as the first criterion, and then, by the highest biodiversity obtained.

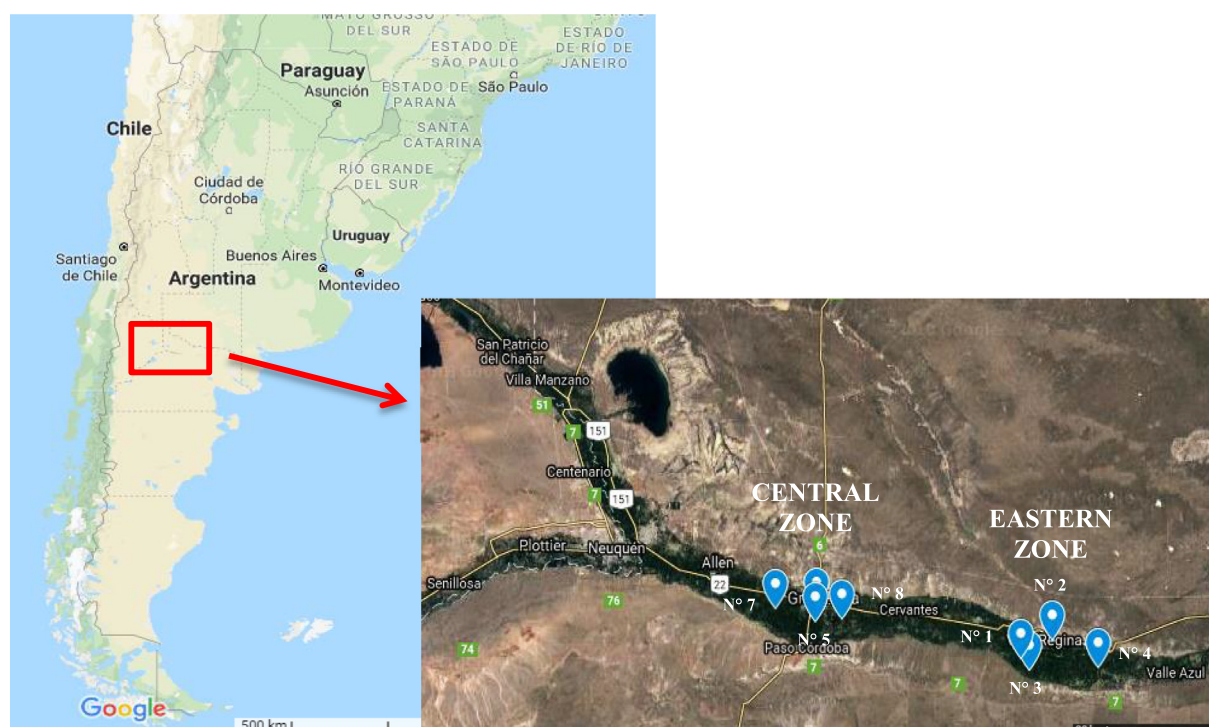


Fig. 1. Map of the agricultural region under study detailing the geographical zones and the eight productive establishments in which the samplings were carried out.

Occasionally, particularly during the summer season, we obtained plates that exceeded 100 CFU/m³. In these cases the count was carried out by quadrants and, therefore, the fungal counts obtained in this study belong to an estimated number.

The sampling frequency was once for each season of the year for three consecutive years between 2014 and 2017. Since the Argentinean Patagonia is characterized by the presence of constant and intense winds, samplings were carried out in days with low wind speed, previously consulting the reference local forecast

provided by the Agrometeorological Observatory belonging to INTA. Samplings of rural settings from each zone were conducted in the same day during the morning hours. Within each rural setting, samples were taken at three geographical points using a GPS to register the coordinates of latitude and longitude of each sampling site. Details of sampling times and weather conditions are described in Table 1.

A total of 288 counting plates were obtained from the samplings conducted in the eight rural settings during the four seasons of each

Table 1
Details of sampling times and weather conditions.

Season	Zone	Date	Weather conditions			
			Temperature (°C)	Wind (Km/h)	Humidity (%)	Global radiation (w/m ²)
AUTUMN 2014	East	26/05	6.8	1.1	75	580
	Central	29/05	10	2.6	68	316
WINTER 2014	East	13/08	1.7	2.1	51	554
	Central	21/08	9.1	2.4	66	401
SPRING 2014	East	12/11	16.6	2.3	43	1022
	Central	05/11	14.8	0.5	60	823
SUMMER 2015	East	23/02	22.1	0.6	63	918
	Central	20/02	19.8	2.3	46	597
AUTUMN 2015	East	04/05	11.9	3.3	56	491
	Central	12/05	9.9	0.8	87	214
WINTER 2015	East	13/08	6.7	1	36	583
	Central	19/08	11.2	4.7	59	386
SPRING 2015	East	10/11	18.6	2.6	ND ^a	ND ^a
	Central	17/11	16.9	10.3	27	676
SUMMER 2016	East	10/02	21.8	0.6	72	954
	Central	11/02	23.2	2.6	74	626
AUTUMN 2016	East	17/05	8.1	0.3	83	509
	Central	23/05	8.4	2.3	87	403
WINTER 2016	East	19/08	8.6	3.7	65	628
	Central	18/08	10.2	16.6	47	397
SPRING 2016	East	23/11	22.7	0.5	60	988
	Central	21/11	16.4	1.1	54	670
SUMMER 2017	East	07/02	22.1	1.1	65	917
	Central	08/02	20.9	1.8	59	665

^a Not determined.

Table 2
Diversity of the fungal genera and species found in each geographical zone.

Eastern zone		Central zone	
Genera	Species	Genera	Species
<i>Acremonium</i>	spp.	<i>Acremonium</i>	spp.
<i>Alternaria</i>	<i>A. alternata</i> <i>A. arborescens</i> <i>A. tenuissima</i> <i>A. sp.</i>	<i>Alternaria</i>	<i>A. alternata</i> <i>A. arborescens</i> <i>A. tenuissima</i> <i>A. sp.</i>
<i>Arthrimum</i>	spp.	<i>Arthrimum</i>	spp.
<i>Aspergillus</i>	<i>A. niger</i> <i>A. versicolor</i>	<i>Aspergillus</i>	<i>A. chevalieri</i> <i>A. niger</i> <i>A. sydowii</i> <i>A. wentii</i> <i>A. sp.</i>
<i>Aureobasidium</i>	spp.	<i>Aureobasidium</i>	spp.
<i>Bipolaris</i>	spp.	<i>Bipolaris</i>	spp.
<i>Botrytis</i>	spp.	<i>Botrytis</i>	spp.
<i>Chrysonilia</i>	spp.		
<i>Cladosporium</i>	<i>C. aggregatocaticratum</i> <i>C. asperulatum</i> <i>C. cladosporioides</i> <i>C. limoniforme</i> <i>C. macrocarpum</i> <i>C. pseudocladosporioides</i> <i>C. ramotenellum</i> <i>C. subtilissimum</i> <i>C. tenellum</i> <i>C. sp.</i>	<i>Cladosporium</i>	<i>C. aggregatocaticratum</i> <i>C. allicinum</i> <i>C. asperulatum</i> <i>C. cladosporioides</i> <i>C. limoniforme</i> <i>C. macrocarpum</i> <i>C. pseudocladosporioides</i> <i>C. ramotenellum</i> <i>C. subtilissimum</i> <i>C. tenellum</i> <i>C. sp.</i>
<i>Curvularia</i>	spp.	<i>Curvularia</i>	spp.
<i>Drechslera</i>	spp.	<i>Drechslera</i>	spp.
<i>Emericella</i>	spp.		
<i>Epicoccum</i>	<i>E. nigrum</i>	<i>Epicoccum</i>	<i>E. nigrum</i>
<i>Fusarium</i>	<i>F. acuminatum</i> <i>F. compactum</i> <i>F. crookwellense</i> <i>F. dlamini</i> <i>F. oxysporum</i> <i>F. poae</i> <i>F. polyphialidicum</i> <i>F. proliferatum</i> <i>F. sambucinum</i> <i>F. semitectum</i> <i>F. sporotrichioides</i> <i>F. sp.</i>	<i>Fusarium</i>	<i>F. acuminatum</i> <i>F. chlamydosporum</i> <i>F. dlamini</i> <i>F. oxysporum</i> <i>F. proliferatum</i> <i>F. reticulatum</i> <i>F. semitectum</i> <i>F. solani</i> <i>F. sporotrichioides</i> <i>F. verticillioides</i> <i>F. sp.</i>
		<i>Geotrichum</i>	
		<i>Moniliella</i>	
		<i>Neosartorya</i>	
<i>Nigrospora</i>	spp.	<i>Nigrospora</i>	spp.
<i>Penicillium</i>	<i>P. brevicompactum</i> <i>P. canescens</i> <i>P. expansum</i> <i>P. paneum</i> <i>P. raistrickii</i> <i>P. solitum</i>	<i>Penicillium</i>	<i>P. brevicompactum</i> <i>P. canescens</i> <i>P. chrysogenum</i> <i>P. expansum</i> <i>P. funiculosum</i> <i>P. janckzewsky</i> <i>P. paneum</i> <i>P. raistrickii</i> <i>P. restrictum</i> <i>P. solitum</i> <i>P. verrucosum</i> <i>P. sp.</i> <i>P. aliena</i> <i>P. betae</i> <i>P. glomerata</i> <i>P. medicaginis</i> <i>P. sp.</i>
<i>Phoma</i>	<i>P. aliena</i> <i>P. glomerata</i> <i>P. sp.</i>	<i>Phoma</i>	<i>P. aliena</i> <i>P. betae</i> <i>P. glomerata</i> <i>P. medicaginis</i> <i>P. sp.</i>
<i>Scopulariopsis</i>	spp.	<i>Scopulariopsis</i>	spp.
		<i>Sordaria</i>	
		<i>Stachybotrys</i>	
<i>Stemphylium</i>	<i>S. vesicarium</i>	<i>Stemphylium</i>	<i>S. vesicarium</i>
<i>Trichoderma</i>	spp.	<i>Trichoderma</i>	spp.
		<i>Trichothecium</i>	spp.

Table 2 (continued)

Eastern zone		Central zone	
Genera	Species	Genera	Species
<i>Ulocladium</i>	spp.	<i>Ulocladium</i>	spp.
		<i>Wallemia</i>	spp.

Non sporulating fungi.
Non identified fungi.

year of study. This value comes from a total of 24 plates per season (3 plates, one for each geographical point of each setting, multiplied by the 8 selected settings) that at the end of each year contributed to a total of 96 plates, which comprised the total of 288 plates obtained during the three years.

2.3. Isolation and morphological identification

Plates obtained after sampling were cultured at 25 °C for 5 days and concentrations were calculated as colony forming units per cubic meter of air (CFU/m³). Differential count of fungal genera was carried out after microscopic observation and macroscopic analysis of colonies in PDA, Malt Extract Agar (MEA) and Water Agar (WA) according to Samson et al. (2000) and Pitt and Hocking (2009). Pure cultures of genera identified from the first two years of sampling were isolated for further identification at species level, while isolates from the third year were limited only to genus identification. 302 isolates of *Cladosporium* were randomly selected and grouped according to the morphological characteristics of the colonies based on mycelia color and diameter, texture, furcation and other general observations of the colony appearance. Furthermore, they were observed microscopically in order to classify them between the three major complexes of this genus based on Bensch et al. (2012, 2015). 59 isolates of *Cladosporium* representative of all the morphological groups obtained were proportionally and randomly chosen for performing molecular analysis (Temperini et al., 2018). 415 monosporic isolates of *Alternaria* were identified based on the determination of group-species according to the sporulation pattern and morphological characteristics of the conidia (Simmons, 2007). 83 monosporic isolates of *Fusarium* were identified using the keys proposed by Nelson et al. (1983) and Leslie and Summerell (2006). 60 isolates of *Penicillium*, 11 isolates of *Aspergillus* and 5 of *Eurotium* were identified according to Pitt and Hocking (2009). 11 isolates of *Stemphylium* were identified according to Kurose et al. (2015) and 20 isolates of *Phoma* according to Boerema (2004). Species of these two last genera were also confirmed by molecular tests.

2.4. Data analysis

The relative density (frequency of appearance) for each genus and species found was calculated according to Smith (1980) by the following equation (Borrego et al., 2011):

$$\text{Relative Density (RD)} = \frac{\text{Number of isolates from a genus or species} \times 100}{\text{Total Number of isolates of all genera or species}}$$

Using this equation, data were organized by annual periods (first, second and third year of sampling) and an Analysis of Variance (ANOVA) followed by Tukey comparison test ($p < 0.05$) were performed in order to determine statistical differences using the InfoStat program (Di Rienzo et al., 2018).

Complete meteorological data from the period 2011–2017 were obtained from the INTA agrometeorological observatory. Temperature, wind speed, humidity and global radiation were the parameters chosen

Table 3Annual mean counts (CFU/m³) and relative densities (%) of the fungal genera found in each year of sampling in each geographical zone.

Genera	Eastern zone					Central zone					Annual average of both zones	
	1st YEAR	2nd YEAR	3rd YEAR	Average of the three years		1st YEAR	2nd YEAR	3rd YEAR	Average of the three years			
	AMC ^a	AMC ^a	AMC ^a	AMC ^a	RD ^b	AMC ^a	AMC ^a	AMC ^a	AMC ^a	RD ^b	AMC ^a	RD ^b
<i>Cladosporium</i>	4901	3303	2496	3567	76.076	3631	2922	3767	3440	77.925	3503	76.973
<i>Alternaria</i>	795	865	423	694	14.807	404	489	433	442	10.009	568	12.480
<i>Epicoccum</i>	105	271	285	221	4.704	137	141	265	181	4.101	201	4.412
<i>Botrytis</i>	5	12	8	8	0.176	89	200	181	157	3.551	83	1.813
<i>Aureobasidium</i>	62	28	30	40	0.847	56	36	58	50	1.133	45	0.986
Non identified fungi	55	23	26	34	0.731	44	90	19	51	1.156	43	0.937
<i>Fusarium</i>	47	47	13	36	0.760	12	29	9	17	0.375	26	0.573
<i>Arthrinium</i>	15	11	23	16	0.347	5	8	14	9	0.206	13	0.279
<i>Drechslera</i>	9	14	14	12	0.260	6	16	16	12	0.278	12	0.269
<i>Phoma</i>	7	4	35	15	0.324	5	8	10	8	0.170	11	0.249
<i>Penicillium</i>	8	25	3	12	0.249	14	10	6	10	0.227	11	0.238
Non sporulating fungi	7	7	14	9	0.201	21	5	6	10	0.237	10	0.218
<i>Ulocladium</i>	4	7	5	5	0.114	15	9	4	9	0.206	7	0.159
<i>Bipolaris</i>	4	14	1	6	0.133	2	5	6	4	0.099	5	0.117
<i>Nigrospora</i>	2	8	1	4	0.076	1	9	ND ^c	4	0.080	4	0.078
<i>Aspergillus</i>	1	1	1	1	0.022	11	2	1	5	0.104	3	0.062
<i>Stemphylium</i>	3	4	1	3	0.053	3	1	2	2	0.038	2	0.046
<i>Curvularia</i>	1	2	1	1	0.031	2	0	1	1	0.027	1	0.029
<i>Acremonium</i>	2	2	ND ^c	1	0.025	1	1	1	1	0.017	1	0.021
<i>Trichoderma</i>	ND ^c	1	1	ND ^c	0.009	ND ^c	2	1	1	0.019	1	0.014
<i>Mucor</i>	1	1	ND ^c	1	0.018	1	ND ^c	ND ^c	ND ^c	0.005	1	0.011
<i>Scopulariopsis</i>	1	ND ^c	ND ^c	ND ^c	0.004	3	ND ^c	ND ^c	1	0.019	1	0.011
<i>Geotrichum</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	1	ND ^c	1	0.014	0 ^d	0.007
<i>Chrysionilia</i>	ND ^c	1	ND ^c	ND ^c	0.009	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	0 ^d	0.005
<i>Trichothecium</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	ND ^c	0.009	0 ^d	0.005
<i>Emericella</i>	ND ^c	1	ND ^c	ND ^c	0.004	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	0 ^d	0.002
<i>Moniliella</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	ND ^c	0.005	0 ^d	0.002
<i>Neosartorya</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	0.005	0 ^d	0.002
<i>Sordaria</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	0.005	0 ^d	0.002
<i>Stachybotrys</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	ND ^c	0.005	0 ^d	0.002
<i>Wallemia</i>	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	1	ND ^c	ND ^c	ND ^c	0.005	0 ^d	0.002

^a Annual mean count.^b Relative densities.^c Not determined.^d These final counts result from the average of the partial counts and their rounding to whole numbers.

to perform the statistical analysis. These data were correlated with fungal mean counts using Pearson correlation coefficient.

2.5. Storage of isolates

Pure isolates of each species identified were maintained on solid MEA and PDA at 4 °C and stored in glycerol 18% v/v at –80 °C for long-term storage.

3. Results

3.1. Diversity of fungal taxa and annual distribution of genera and species

A great fungal diversity was found in the rural environments of the region in each year of sampling. At least 28 genera and 50 species were determined. In the central zone it was registered a wider diversity of fungal genera and species (Table 2).

In both geographical zones, the predominant genus was *Cladosporium* followed by *Alternaria* and *Epicoccum* (Table 3) and the most frequent species was *C. cladosporioides* followed by *C. limoniforme* (Table 4).

Anova test followed by Tukey comparison test ($p < 0.05$) showed statistical differences in *Cladosporium* and *Alternaria* genera. *C. cladosporioides* and *C. limoniforme* did not show statistical differences between them, but both were statistically different to the other species of the genus. In the same way, *Alternaria tenuissima* showed statistical differences with the other *Alternaria* species. No statistical differences

were found for *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., and *Phoma* spp.

3.2. Annual fungal counts

The annual mean count (AMC) of viable fungal spores remained in the order of E+04 in the three years of sampling in each geographical zone (Table 5). Anova test followed by Tukey comparison test ($p < 0.05$) were used to compare the AMC between both geographical zones. There were no significant differences ($p > 0.05$) between the AMC of each geographical zone. There were no significant differences ($p > 0.05$) between the average AMC of both geographical zones.

3.3. Seasonal fungal counts

Anova test followed by Tukey comparison test ($p < 0.05$) were used to compare seasons within each year of sampling (Table 6). Significant differences were found only between the mean summer count with respect to the other seasons in the first and third year. In the second year, significant differences ($p < 0.05$) were determined only between the mean counts of the autumnal season and the spring season, which presented the highest and the lowest fungal count respectively.

Statistical analysis was also used to find yearly differences restricted to a specific season. No significant differences were found for mean counts in autumn, spring and summer seasons, during the three-year sampling period. Nevertheless, significant differences

Table 4Annual mean counts (CFU/m³) and relative densities (%) of the fungal species found in each year of sampling in each geographical zone.

Species	Eastern zone				Central zone				Annual average of both zones	
	1st YEAR	2nd YEAR	Average of the two years		1st YEAR	2nd YEAR	Average of the two years			
	AMC ^a	AMC ^a	AMC ^a	RD ^b	AMC ^a	AMC ^a	AMC ^a	RD ^b	AMC ^a	RD ^b
<i>Cladosporium cladosporioides</i>	1934	1649	1791	33.522	1877	1201	1539	36.467	1665	34.822
<i>Cladosporium limoniforme</i>	2022	551	1286	24.075	1084	498	791	18.745	1039	21.723
<i>Alternaria tenuissima</i>	649	656	653	12.212	353	434	394	9.328	523	10.939
<i>Cladosporium asperulatum</i>	449	469	459	8.587	278	529	403	9.552	431	9.013
<i>Other species</i>	169	130	150	2.799	248	382	315	7.468	232	4.859
<i>Cladosporium pseudocladosporioides</i>	10	261	136	2.539	4	412	208	4.921	172	3.590
<i>Epicoccum nigrum</i>	105	271	188	3.524	137	141	139	3.294	164	3.423
<i>Cladosporium subtilissimum</i>	308	37	173	3.231	96	13	55	1.294	114	2.376
<i>Alternaria alternata</i>	93	173	133	2.480	24	37	30	0.718	81	1.702
<i>Cladosporium aggregatocitricatatum</i>	21	111	66	1.241	21	171	96	2.269	81	1.694
<i>Cladosporium tenellum</i>	126	180	153	2.867	ND ^c	17	9	0.202	81	1.691
<i>Cladosporium allicinum</i>	ND ^c	ND ^c	ND ^c	0.000	203	ND ^c	101	2.403	51	1.060
<i>Cladosporium sp.</i>	31	35	33	0.614	47	40	43	1.029	38	0.797
<i>Alternaria sp.</i>	54	12	33	0.614	25	18	21	0.506	27	0.567
<i>Fusarium sporotrichioides</i>	21	31	26	0.480	ND ^c	9	5	0.111	15	0.317
<i>Cladosporium ramotenellum</i>	ND ^c	8	4	0.075	11	32	22	0.510	13	0.267
<i>Fusarium semitectum</i>	9	2	6	0.105	6	14	10	0.244	8	0.167
<i>Alternaria arborescens</i>	ND ^c	24	12	0.228	2	ND ^c	1	0.018	6	0.135
<i>Cladosporium macrocarpum</i>	ND ^c	2	1	0.022	10	9	9	0.224	5	0.111
<i>Penicillium brevicompactum</i>	ND ^c	10	5	0.094	6	3	5	0.111	5	0.101
<i>Penicillium canescens</i>	3	13	8	0.146	1	ND ^c	ND ^c	0.007	4	0.085
<i>Stemphylium vesicarium</i>	3	4	3	0.064	3	1	2	0.037	3	0.052
<i>Phoma aliena</i>	4	2	3	0.053	3	1	2	0.044	2	0.049
<i>Fusarium acuminatum</i>	ND ^c	8	4	0.070	ND ^c	1	1	0.019	2	0.048
<i>Penicillium expansum</i>	3	ND ^c	1	0.023	5	1	3	0.067	2	0.042
<i>Phoma glomerata</i>	ND ^c	1	ND ^c	0.006	1	5	3	0.074	2	0.036
<i>Fusarium proliferatum</i>	4	2	3	0.050	1	ND ^c	1	0.012	2	0.033
<i>Phoma sp.</i>	3	1	2	0.041	1	1	1	0.015	1	0.029
<i>Fusarium crookwellense</i>	5	ND ^c	3	0.047	ND ^c	ND ^c	ND ^c	0.000	1	0.026
<i>Fusarium sp.</i>	3	ND ^c	1	0.023	2	1	1	0.030	1	0.026
<i>Fusarium compactum</i>	4	ND ^c	2	0.041	ND ^c	ND ^c	ND ^c	0.000	1	0.023
<i>Aspergillus chevalieri</i>	ND ^c	ND ^c	ND ^c	0.000	4	ND ^c	2	0.044	1	0.020
<i>Fusarium polyphialidicum</i>	ND ^c	3	2	0.029	ND ^c	ND ^c	ND ^c	0.000	1	0.016
<i>Penicillium paneum</i>	ND ^c	2	1	0.018	ND ^c	1	1	0.015	1	0.016
<i>Aspergillus niger</i>	ND ^c	1	ND ^c	0.006	1	1	1	0.024	1	0.014
<i>Penicillium funiculosum</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	3	1	0.030	1	0.013
<i>Penicillium raistrickii</i>	1	1	1	0.018	ND ^c	1	ND ^c	0.007	1	0.013
<i>Aspergillus versicolor</i>	1	1	1	0.018	ND ^c	ND ^c	ND ^c	0.000	0 ^d	0.010
<i>Aspergillus wentii</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	1	0.015	0 ^d	0.007
<i>Fusarium oxysporum</i>	ND ^c	1	ND ^c	0.006	ND ^c	1	ND ^c	0.007	0 ^d	0.007
<i>Fusarium poae</i>	1	ND ^c	1	0.012	ND ^c	ND ^c	ND ^c	0.000	0 ^d	0.007
<i>Fusarium reticulatum</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	1	0.015	0 ^d	0.007
<i>Fusarium sambucinum</i>	ND ^c	1	1	0.012	ND ^c	ND ^c	ND ^c	0.000	0 ^d	0.007
<i>Fusarium solani</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	1	0.015	0 ^d	0.007
<i>Penicillium restrictum</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	1	0.015	0 ^d	0.007
<i>Penicillium solitum</i>	1	ND ^c	ND ^c	0.006	ND ^c	1	ND ^c	0.007	0 ^d	0.007
<i>Fusarium dlamini</i>	ND ^c	ND ^c	ND ^c	0.004	1	ND ^c	ND ^c	0.009	0 ^d	0.006
<i>Aspergillus sp.</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	1	0.012	0 ^d	0.005
<i>Aspergillus sydowii</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	ND ^c	0.007	0 ^d	0.003
<i>Fusarium chlamydosporum</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003
<i>Fusarium verticillioides</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003
<i>Penicillium chrysogenum</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	ND ^c	0.007	0 ^d	0.003
<i>Penicillium janckzewsky</i>	ND ^c	ND ^c	ND ^c	0.000	1	ND ^c	ND ^c	0.007	0 ^d	0.003
<i>Penicillium verrucosum</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003
<i>Penicillium sp.</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003
<i>Phoma betae</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003
<i>Phoma medicaginis</i>	ND ^c	ND ^c	ND ^c	0.000	ND ^c	1	ND ^c	0.007	0 ^d	0.003

^a Annual mean count.^b Relative densities.^c Not determined.^d These final counts result from the average of the partial counts and their rounding to whole numbers.

were found for the 3 winter seasons: the second year was significantly different to the third year (with p-value = 0.0011). Meteorological data were also analyzed in order to detect any change on weather factors during the three sampled winters that could explain this difference. As a result, there were statistical differences in the mean humidity percentage between winter of 2015 and winter of

2016 (p-value = 0.027). The winter of 2016 had a mean humidity percentage of 66.2%, while 2015 was 50.0% (as a reference 2014 winter was 59.8%). In addition, the same weather factors were analyzed for the extended period of 2011–2017, and again the only statistical difference found was mean humidity percentage between the winter season of 2015 and winter season of 2016.

Table 5Annual counts (CFU/m³) determined in both geographical zones.

	1st YEAR			2 nd YEAR			3 rd YEAR		
	Eastern zone	Central zone	Average	Eastern zone	Central zone	Average	Eastern zone	Central zone	Average
Mean	6.04E+03	4.46E+03	5.25E+03	4.65E+03	3.98E+03	4.32E+03	3.38E+03	4.80E+03	4.09E+03
Minimum	1.30E+03	1.96E+03	1.63E+03	2.35E+03	2.50E+03	2.42E+03	2.26E+03	3.18E+03	2.72E+03
Maximum	9.82E+03	1.00E+04	9.92E+03	8.13E+03	6.52E+03	7.32E+03	3.97E+03	6.03E+03	5.00E+03
Standard deviation	3.93E+03	3.74E+03	3.84E+03	2.49E+03	1.75E+03	2.12E+03	7.82E+02	1.19E+03	9.84E+02

3.4. Correlation between meteorological data and annual mean counts

When Pearson correlation coefficient was applied to correlate mean fungal counts and weather factors (average values for the season, and specifically for the day of sampling) no correlation was found.

4. Discussion

The information obtained from the present work provides knowledge of the fungal genera and species present in the air of agricultural environments of a cold dry desert climate. This is the first study in our country and the first one in a rural area with this type of climate and land use whose results make a valuable contribution.

A wide diversity of fungi was found with >28 genera and 50 species determined through the whole sampling period. Among them, species of *Cladosporium* and *Alternaria* were the most abundant as other researchers previously reported in rural environments (Pepelnjak and Šegvič, 2003; Kasprzyk and Worek, 2006; Oliveira et al., 2010). Furthermore, *C. cladosporioides*, *C. limoniforme* and *A. tenuissima* showed significantly higher abundances than the rest of the species of each respective genus. Initially, these species could be considered as a bio-indicator of environmental stress and change, however, we consider that the information found so far is not enough to propose any of them as such. As species from the same genus can have different requirements for sporulation and growth, additional studies of ecophysiology would be needed to determine if it is possible. Moreover, it would be necessary to increase the number of sampling in each season and the time of the study might also need to be increased (sampling during a longer period than three years). Since the study area is dedicated to fruit growth and has a considerable population, these genera become important due to their health and agricultural implications (Abdel Hameed et al., 2009; Reyes et al., 2009; Oliveira et al., 2010). In fact, among the phytopathogenic fungi isolated from air capable of causing disease in pip fruits of this study area, *Alternaria* diseases (black spot on leaves, rot of fruits and Moldy heart) were present during the three summers, which is consistent with the maximum counts obtained in the summer season of the three years. When weather conditions are favorable, these pathogens can increase the severity of pathologies. In the Middle Valley of Río Negro, a nearby region of the study area, changes in climatological conditions (raise of rainfalls and humidity) during 2013–2014 increased the severity of walnut apical necrosis caused by microbial complexes including *Alternaria* (Temperini et al., 2017). Regarding *Cladosporium*, the rot in pears caused by species of the *C. herbarum* complex in cold long-term storage acquired relevance during 2015, which could be related to the

maximum count found for this genus at the summer season of this year. The rest of the genera remained in lower concentrations even at relative densities lower than 1%. However, it is important to report their presence, since their ability to tolerate the unique conditions of this study area may reveal their ability to also overcome and adapt to climate change effects and develop or enhance their pathogenic capacity. In fact, *S. vesicarium* is another field pathogen of interest in this area, especially during the summer season of 2017, where weather conditions favored its development and pathogenic capacity (Llorente et al., 2012) affecting mainly leaves and fruits of pear cultivars. These results reveal that weather parameters may affect the aeromycoflora behavior and its phytopathological implications. Indeed, the concentration and type of fungal taxa present in the atmosphere are influenced by the geographical and climatic characteristics of a region which determine the predominant type of vegetation, decomposing organic matter and agricultural activity (Awad, 2005; Sánchez Espinosa and Almaguer Chávez, 2014). Therefore, in this context, the concept of climate change becomes relevant. Climate predictions indicate an increase in drought events in summer providing the environmental dryness needed for conidia release into the atmosphere, thus airborne fungal concentrations are expected to be increased in future years (Maya-Manzano et al., 2016; Sindt et al., 2016).

Even though no significant differences were found between the AMC of each geographical zone, the composition of fungal taxa and their abundance vary discreetly, with the exception of the three predominant genera (*Cladosporium*, *Alternaria* and *Epicoccum*) and four predominant species (*C. cladosporioides*, *C. limoniforme*, *A. tenuissima*, *C. asperulatum*). These results could be related to the similarities of geographical characteristics and the short distance that separates both zones. On the other hand, while no statistical differences were determined between the average AMC of both geographical zones, it was revealed a different seasonality of fungal counts between the first and third year compared to the second one. Moreover, statistical differences were found between winter of 2015 and 2016. However, no correlation was found between weather parameters and mean counts. In this context, the number of environmental factors that affect the daily and seasonal rhythms of the occurrence of airborne fungal spores are known to but it remains difficult to estimate the relative importance of each factor (Kasprzyk and Worek, 2006). Therefore these differences may arise from a combination of factors including organic matter, changes in vegetation, agricultural and/or human activities which dynamically change in this rural valley due to economic situation that the productive sector has been going through the past years which has led to the replacement of fruit fields by other types of farmlands or, even, different kind of economical entrepreneurship.

Table 6Seasonal counts (CFU/m³) determined in each year of sampling.

	1st YEAR				2nd YEAR				3rd YEAR			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Mean	3.02E+03	2.24E+03	3.06E+03	1.27E+04	7.30E+03	3.42E+03	1.07E+03	5.49E+03	3.44E+03	7.48E+02	2.56E+03	9.61E+03
Minimum	9.30E+02	7.80E+02	5.50E+02	1.21E+03	2.36E+03	2.12E+03	4.50E+02	1.69E+03	9.70E+02	6.80E+02	4.80E+02	4.64E+03
Maximum	9.67E+03	4.52E+03	6.60E+03	2.91E+04	2.50E+04	5.77E+03	2.84E+03	1.70E+04	6.91E+03	9.00E+02	5.94E+03	1.69E+04
Standard deviation	2.83E+03	1.71E+03	2.23E+03	1.15E+04	7.37E+03	1.50E+03	7.94E+02	4.87E+03	2.21E+03	7.01E+01	2.02E+03	4.30E+03

5. Conclusions

The study area comprises a region with unique geographical and climatic characteristics due to its aridity, dryness, constant intense winds and coexistence and proximity of population nuclei to agricultural settings, and its wide fungal diversity has been able to adapt to these environmental conditions. Moreover, the fungal flora and its concentration have been consistent through the three-year period. Even though these results make up the first report of the area and we cannot compare them to previous data, they raise the possibility of fungal taxa to adapt to climate change and remain present in the atmosphere with their potential consequent effects on human, animal and plant health. Moreover, due to the particular characteristics of this study area, climate change may eventually turn it into an environment which could be optimum for the development and prevalence of extremophile fungi.

In this way, the knowledge generated will allow the use of adequate and efficient methods to reduce the biological and economic damage of fungal taxa, evaluate the potential mycotoxicological risk and, in the context of climate change, predict fungal flora fluctuations and emerging diseases caused by potential pathogenic microorganisms that have not been reported to cause damage at the present.

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Conflict of interest

None.

References

- Abdel Hameed, A.A., Khoder, M.I., Yuosra, S., Osman, A.M., Ghanem, S., 2009. Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. *Sci. Total Environ.* 407, 6217–6222.
- Awad, A.H.A., 2005. Vegetation: a source of air fungal bio-contaminant. *Aerobiologia* 21 (1), 53–61.
- Beggs, P.J., 2010. Adaptation to impacts of climate change on aeroallergens and allergic respiratory diseases. *Int. J. Environ. Res. Public Health* 7 (8), 3006–3021.
- Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W., 2012. The genus *Cladosporium*. *Stud. Mycol.* 72, 1–401.
- Bensch, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., de Jesús Yáñez-Morales, M., Crous, P.W., 2015. Common but different: the expanding realm of *Cladosporium*. *Stud. Mycol.* 82, 23–74.
- Boccacci, L., Casilli, M., Grandi, C., D'Ovidio, M.C., Pace, L., 2017. Monitoring Airborne Fungal Spores (*Alternaria* and *Cladosporium*) in L'Aquila: A Useful Tool for Climate Change and Occupational Health Assessment. <http://www.dispaa.unifi.it/vp-555-pubblicazioni.html>.
- Boerema, G.H., 2004. *Phoma Identification Manual: Differentiation of Specific and Infra-specific Taxa in Culture*. first ed. CABI.
- Borrego, S.C., Perdomo, I., de la Paz, J., de Saravia, S.G.G., Guimet, P.S., 2011. Relevamiento microbiológico del aire y de materiales almacenados en el Archivo Histórico del Museo de La Plata, Argentina y en el Archivo Nacional de la República de Cuba. *Revista del Museo de la Plata* 18 (119), 1–18.
- Cabral, J.P., 2010. Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. *Sci. Total Environ.* 408 (20), 4285–4295.
- Curtis, L., Rea, W., Smith-Willis, P., Fenyves, E., Pan, Y., 2006. Adverse health effects of outdoor air pollutants. *Environ. Int.* 32 (6), 815–830.
- De la Rosa, M., Mosso, M., Ullán, C., 2002. El aire: hábitat y medio de transmisión de microorganismos. *Observatorio medioambiental* 5, 375–402.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., InfoStat versión, 2018. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL: <http://www.infostat.com.ar>.
- Fröhlich-Nowoisky, J., Pickersgill, D.A., Després, V.R., Pöschl, U., 2009. High diversity of fungi in air particulate matter. *Proc. Natl. Acad. Sci.* 106 (31), 12814–12819.
- Fröhlich-Nowoisky, J., Burrows, S.M., Xie, Z., Engling, G., Solomon, P.A., Fraser, M.P., Mayol-Bracero, O.L., Artaxo, P., Begerow, D., Conrad, R., Andreae, M.O., Després, V.R., Pöschl, U., 2012. Biogeography in the air: fungal diversity over land and oceans. *Biogeosciences* 9 (3), 1125.
- INDEC, 2010. Censo Nacional de Población. Hogares y Viviendas 2010. https://www.indec.gov.ar/nivel4_default.asp?id_tema_1=2&id_tema_2=41&id_tema_3=135.
- Isard, S.A., Russo, J.M., Ariatti, A., 2007. The integrated aerobiology modeling system applied to the spread of soybean rust into the Ohio River valley during September 2006. *Aerobiologia* 23 (4), 271–282.
- Kakde, U.B., 2012. Fungal bioaerosols: global diversity, distribution and its impact on human beings and agricultural crops. *Bionano Front.* 5 (0974-0678).
- Kasprzyk, I., 2008. Aeromycology—main research fields of interest during the last 25 years. *Ann. Agric. Environ. Med.* 15 (1), 1–7.
- Kasprzyk, I., Worek, M., 2006. Airborne fungal spores in urban and rural environments in Poland. *Aerobiologia* 22 (3), 169.
- Khan, A.H., Karuppaiyil, S.M., 2012. Fungal pollution of indoor environments and its management. *Saudi J. Biol. Sci.* 19 (4), 405–426.
- Köppen, W., 1931. *Grundriss der Klimakunde*. Walter de Gruyter, Berlin.
- Kurose, D., Misawa, T., Suzui, T., Ichikawa, K., Kisaki, G., Hoang, L.H., Furuya, N., Tsuchiya, K., Tsushima, S., Sato, T., 2015. Taxonomic re-examination of several Japanese *Stemphylium* strains based on morphological and molecular phylogenetic analyses. *J. Gen. Plant Pathol.* 81 (5), 358–367.
- Lang-Yona, N., Shuster-Meiseles, T., Mazar, Y., Yarden, O., Rudich, Y., 2016. Impact of urban air pollution on the allergenicity of *Aspergillus fumigatus* conidia: outdoor exposure study supported by laboratory experiments. *Sci. Total Environ.* 541, 365–371.
- Lanier, C., Richard, E., Heutte, N., Picquet, R., Bouchart, V., Garon, D., 2010. Airborne molds and mycotoxins associated with handling of corn silage and oilseed cakes in agricultural environment. *Atmos. Environ.* 44 (16), 1980–1986.
- Leslie, J.F., Summerell, B.A., 2006. *The Fusarium Laboratory Manual*. first ed. Blackwell Publishing.
- Llorente, I., Moragrega, C., Ruz, L., Montesinos, E., 2012. An update on control of brown spot of pear. *Trees* 26 (1), 239–245.
- Maya-Manzano, J.M., Muñoz-Triviño, M., Fernandez-Rodríguez, S., Silva-Palacios, I., Gonzalo-Garjón, A., Tormo-Molina, R., 2016. Airborne *Alternaria* conidia in Mediterranean rural environments in SW of Iberian Peninsula and weather parameters that influence their seasonality in relation to climate change. *Aerobiologia* 32 (1), 95–108.
- Mentese, S., Rad, A.Y., Arisoy, M., Güllü, G., 2012. Seasonal and spatial variations of bioaerosols in indoor urban environments, Ankara, Turkey. *Indoor Built Environ.* 21 (6), 797–810.
- Muhsin, T.M., Adlan, M.M., 2012. Seasonal distribution pattern of outdoor airborne fungi in Basrah city, southern Iraq. *J. Basrah Res.* 38, 90–98.
- Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium Species: An Illustrated Manual for Identification*. Penn State University Press.
- Newbound, M., McCarthy, M.A., Lebel, T., 2010. Fungi and the urban environment: a review. *Landsc. Urban Plan.* 96 (3), 138–145.
- Oliveira, M., Guerner-Moreira, J., Mesquita, M.M., Abreu, I., 2009. Important phytopathogenic airborne fungal spores in a rural area: incidence of *Botrytis cinerea* and *Oidium* spp. *Ann. Agric. Environ. Med.* 16 (2), 197–204.
- Oliveira, M., Ribeiro, H., Delgado, L., Fonseca, J., Gastel-Branco, M.G., Abreu, I., 2010. Outdoor allergenic fungal spores: comparison between an urban and a rural area in northern Portugal. *J. Investig. Allergol. Clin. Immunol.* 20 (2), 117.
- Pepejnjak, S., Šegvić, M., 2003. Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia. *Aerobiologia* 19 (1), 11–19.
- Pitt, J., Hocking, A., 2009. *Fungi and Food Spoilage*. third ed. Springer.
- Reyes, E.S., de la Cruz, D.R., Merino, E.S., Sánchez, J.S., 2009. Meteorological and agricultural effects on airborne *Alternaria* and *Cladosporium* spores and clinical aspects in Valladolid [Spain]. *Ann. Agric. Environ. Med.* 16 (1), 53–61.
- Rodríguez, A., Muñoz, A., 2006. Síntesis Agrometeorológica para el periodo 1990–2004. *Boletín Divulgación Técnica* 53, 38.
- Samson, R., Hoekstra, E., Frisvad, J., Filtenborg, O., 2000. *Introduction to Food- and Airborne Fungi*. sixth ed. Amer. Society for Microbiology.
- Sánchez Espinosa, K.C., Almaguer Chávez, M., 2014. Aeromicrología y salud humana. *Rev. Cubana Med. Trop.* 66 (3), 322–337.
- SENASA, 2017. Anuario Estadístico 2016. Centro Regional Patagonia Norte http://www.senasa.gob.ar/sites/default/files/ARBOL_SENESA/SENASA%20COMUNICA/adjuntos_varios/anuario_estadistico_2016_crpn.pdf.
- Sharpe, R.A., Bearman, N., Thornton, C.R., Husk, K., Osborne, N.J., 2015. Indoor fungal diversity and asthma: a meta-analysis and systematic review of risk factors. *J. Allergy Clin. Immunol.* 135 (1), 110–122.
- Shelton, B.G., Kirkland, K.H., Flanders, W.D., Morris, G.K., 2002. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol.* 68 (4), 1743–1753.
- Simmons, E.G., 2007. *Alternaria: An Identification Manual*. first ed. ASM Press.
- Sindt, C., Besancenot, J.P., Thibaudon, M., 2016. Airborne *Cladosporium* fungal spores and climate change in France. *Aerobiologia* 32 (1), 53–68.
- Smith, G., 1980. *Ecology and Field Biology*. third ed. Harper & Row.
- Temperini, C.V., Pardo, A.G., Pose, G.N., 2017. First report of apical necrosis in walnut cultivars from northern Argentinean Patagonia. *J. Plant Pathol. Microbiol.* 8, 414. <https://doi.org/10.4172/2157-7471.1000414>.
- Temperini, C.V., Pardo, A.G., Pose, G.N., 2018. Diversity of airborne *Cladosporium* species isolated from agricultural environments of northern Argentinean Patagonia: molecular characterization and plant pathogenicity. *Aerobiologia* 1–13.